Science of the Total Environment 628–629 (2018) 1009–1016

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of rice-straw biochar on nitrous oxide emissions from paddy soils under elevated CO_2 and temperature

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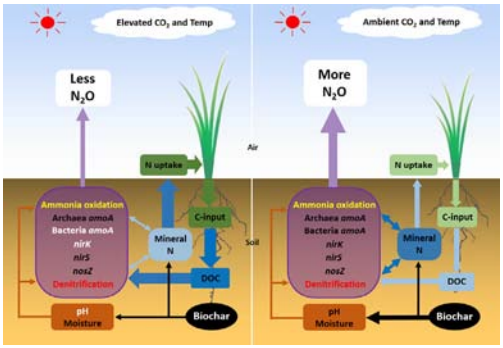
HIGHLIGHTS

- N_2O emissions was suppressed under eCO_2 and eTemp.
- The stimulating effect of biochar on N_2O emissions was attenuated under eCO_2 and eTemp.
- Reduced mineral N and increased DOC could explain the decreased N_2O emissions at eCO_2 and eTemp.
- Increased soil moisture and pH with biochar amendment could influence the microbial N cycle.
- The lessened liming effect of biochar might explain the attenuated stimulating effect.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form 7 January 2018
Accepted 4 February 2018
Available online 20 February 2018

Keywords:
Elevated CO_2
Elevated temperature
Ammonia oxidation
Denitrification
Nitrogen cycle

ABSTRACT

In paddy ecosystems, the feedback of N_2O emissions on climate change as well as the effects of biochar on N_2O emissions will have a profound impact on global warming and rice production. In order to investigate the effect of biochar amendment on N_2O emissions from paddy soils under elevated atmospheric CO_2 concentration (700 ppm) and air temperature (ambient +3 °C), a microcosm study was performed in plant growth chambers with rice plant and rice-straw-derived biochar. N_2O emissions from urea-fertilized paddy soils had a significant increase with the biochar amendment during midseason drainage under ambient CO_2 concentration and air temperature. However, N_2O emissions was suppressed under simultaneous elevated CO_2 concentration and air temperature, regardless of whether biochar was amended; under this condition the stimulating effect of biochar was attenuated. Reduced mineral N concentrations and increased DOC concentrations could inhibit N_2O emission at simultaneously elevated CO_2 concentration and air temperature. Increased soil pH and variation in the abundance of archaeal and bacterial *amoA* genes indicated that biochar amendment could stimulate ammonia oxidation-induced N_2O emission during nitrification. Moreover, the liming effect of biochar was lessened under elevated CO_2 concentration and temperature, which may contribute to the attenuated stimulating effect of biochar on N_2O emissions. More attention should to be paid to the effect of soil pH-induced changes in ammonia oxidation, when mitigating N_2O emissions in urea-fertilized paddy soils with biochar amendment at elevated CO_2 concentrations and air temperatures.

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https://doi.org/10.1016/j.scitotenv.2018.02.046
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1. Introduction

Nitrous oxide is a long-lived potent greenhouse gas with almost 300-fold greater global warming potential in comparison to carbon dioxide (Thomson et al., 2012). N_2O is responsible for approximately 6 to 8% of the current global warming (Smith et al., 2007). N_2O also acts as an important catalyst for stratospheric ozone depletion (Ravishankara et al., 2009). Atmospheric N_2O concentration has already reached 324 ppb with an increase of 20% in the past 200 years, and it is predicted to continue increasing (Stocker et al., 2013). Agricultural activities are estimated to contribute as much as 60% of global anthropogenic N_2O emissions (Denman and Brasseur, 2007).

N_2O emissions from rice cultivation account for approximately 11% of global agricultural N_2O emissions (Chapa, 2011). Water regimes in paddy fields are of major importance in regulating nitrogen fertilizer-induced direct N_2O emissions (Zou et al., 2009). It has been well demonstrated that substantial amounts of N_2O can be emitted from paddy fields during midseason drainage and alternate wet–dry irrigation (Cai et al., 1997; Zou et al., 2005; Zheng et al., 2000). These regimes have proved to be effective options for mitigating CH_4 emissions (Cai et al., 1997; Li et al., 2011). Mean N_2O emissions during the rice-cropping season are 993 ± 1075 g (N) ha^{-1} $season^{-1}$ for fertilized fields with midseason drainage (Akiyama et al., 2005). Direct N_2O emissions during the rice growing season amount to 29.0 Gg N_2O-N, which accounts for 7 to 11% of the reported estimates of annual total emissions from croplands in mainland China (Zou et al., 2007). To meet the increasing demand for rice, larger amounts of N fertilizer must be applied, which is likely to increase paddy N_2O emissions.

The CO_2 concentration in the atmosphere has risen by 40% since 1750 to 400 ppm, and the global average surface air temperature has increased by approximately 0.72 °C since 1950 (Stocker et al., 2013). The CO_2 level is predicted to be 700 to 1000 ppm by 2100, and further warming by 2.6 to 4.8 °C is expected during this century (Society and Sciences, 2014). The microbial nitrogen cycle in paddy soils, as an essential part of the global nitrogen cycle, is sensitive to climate change. Previous studies on the impact of climate change on the nitrogen cycle in paddy soils have mainly focused on nitrogen dynamics, soil enzymatic activities, and microbial populations (Cheng et al., 2006; Bhattacharyya et al., 2013). Although N_2O emissions from paddy soils are not as huge as CH_4 emissions, the contribution of N_2O emissions from paddy soils to climate change should be a matter of concern, because it could have a profound influence on the nitrogen cycle in the future. A meta-analysis study revealed that increased atmospheric CO_2 (ranging from 463 to 780 ppm) could stimulate N_2O emissions by 18.8% (van Groenigen et al., 2011). The effects of elevated CO_2 concentration in combination with N fertilization may intensify N_2O emissions (Dijkstra et al., 2012). A recent study reported that N_2O emissions from tropical flooded rice fields significantly increased by 21.4 to 24.6% under elevated CO_2 concentration and temperature (Bhattacharyya et al., 2013). Appropriate strategies for paddy N_2O mitigation are required to minimize the contribution of rice production to global warming.

Biochar is a carbon-rich solid produced during the pyrolysis of biomass in the absence of oxygen (Lehmann, 2007). Rice-straw derived biochar has attracted considerable attention because of its great potential to improve soil quality, increase rice yield, and mitigate the contribution to global warming of paddy rice cultivation (Liu et al., 2011; Dong et al., 2013; Dong et al., 2015; Zhao et al., 2014; Shen et al., 2014; Wang et al., 2017). CH_4 emissions from paddy soil were reduced significantly by 39.5% with rice-straw biochar amendment under elevated CO_2 concentration and air temperature (Han et al., 2016). However, the effect of this biochar on N_2O emissions from paddy soils under elevated atmospheric CO_2 concentration and air temperature remains uncertain. This hinders evaluation of the potential role of biochar amendment in paddy ecosystems to combat climate change in the future.

The objective of this study was to investigate the effect of rice-straw biochar on N_2O emissions from paddy soils under elevated CO_2 concentration and air temperature. The following hypotheses were proposed. (1) Elevated CO_2 concentration and air temperature could affect soil properties by influencing plant growth. (2) Biochar amendment could shift soil properties. (3) Changes in soil properties could have a great impact on the activity and abundance of functional microorganisms associated with the nitrogen cycle. (4) Soil N_2O emissions would change due to the changed microbial nitrogen cycle. To test these hypotheses, four sets of microcosm experiments were conducted. Rice plant growth was monitored and soil chemical properties were measured. The abundance of functional genes associated with the microbial nitrogen cycle (archaeal and bacterial *amoA* genes, *nirS* and *nirK* genes, and *nosZ* gene) were quantified.

2. Materials and methods

2.1. Microcosm study

The soil used in this study was collected from the 0- to 15-cm layer of a rice field in Yuhang District (119.5°E, 30.2°N), Hangzhou, Zhejiang Province, China. This site is a traditional and dominant rice production area. The biochar, made from rice straw, was produced under an oxygen-free atmosphere in a nitrogen-filled chamber through slow pyrolysis (at a heating rate of 5 °C/min^{-1}) at 500 °C with a mean residence time of 3 h. The experimental soil had a pH of 5.09 (1:2.5 H_2O w/v), an EC of 0.07 ms cm^{-1}, the total C of 22.1 g kg^{-1} and total N of 2.7 g kg^{-1}. The biochar used in this study had a pH of 8.88 (1:10 H_2O w/v), an EC of 0.61 ms cm^{-1}, the total C of 551.8 g kg^{-1} and total N of 14.2 g kg^{-1}.

Microcosm experiments were conducted in the CO_2 facility at the Agricultural Experiment Station of Zhejiang University, Hangzhou, China. This was a split–split plot experiment with whole plots randomly assigned to each of four groups. The whole-plot treatments were two biochar application rates (CK, added level at 0; BC, added level at 2.5% w/w). The split-plot factors were two atmospheric CO_2 levels (ambient CO_2 390 ± 10 ppm (aCO_2) and elevated CO_2 700 ± 10 ppm (eCO_2)), which were further split into two air temperature levels (ambient temperature (aTemp): average air temperature of April to July in Hangzhou and elevated temperature (eTemp): ambient level + 3 °C). Thus, there were four groups of plots and eight treatments (aCO_2 and aTemp (aaCK, aaBC); aCO_2 and eTemp (aeCK, aeBC); eCO_2 and aTemp (eaCK, eaBC); eCO_2 and eTemp (eeCK, eeBC)). There were four replicates for each treatment.

Three rice strains (*Oryza sativa* L, Zhongzao 39) with the same growth trend were transferred to a pot (30 cm high, 20 cm inner diameter) with a 5.0 kg matrix (soils with or without biochar) after seeding pre-incubation. Each pot initially received 60 mg of N kg^{-1} (urea), 100 mg of P_2O_5 kg^{-1} (KH_2PO_4), and 100 mg of K_2O kg^{-1} (KCl), with an additional 45 mg of N kg^{-1} (urea) at the tillering phase (18 DAT) and heading phase (82 DAT), respectively. A water regime following the sequence of flooding–midseason drainage–reflooding–moist without water logging (F–D–F–M) was practiced in this study. These microcosm experiments were described in greater detail in our previous report (Han et al., 2016).

2.2. Rice plant growth monitoring and soil properties analysis

Before harvest, the growth status of the rice plants was observed through plant height monitoring. After harvest, the rice plant samples, separated into above- and below-ground parts, were oven-dried at 80 °C for 72 h for measurement of the dry matter weight of the plant biomass (Tokida et al., 2013). Rhizosphere soil samples were collected during the early-midseason drainage (30 DAT), at the bottom of the rice stems within a 5 cm diameter and at an average depth of about 15 cm. Duplicate soil samples were taken from each pot. Samples for chemical measurements and molecular analysis were kept at 4 °C and −70 °C,

respectively. Soil moisture concentration was determined by weight loss after drying at 105 °C for longer than 24 h. Soil pH and electrical conductivity (EC) were determined in 1:2.5 (w/v) water extracts. NH_4^+-N, NO_2^--N and NO_3^--N were extracted from fresh soil with 2.0 M KCl at 1:10 (w/v) after sampling and quantified with colorimetric methods (Mulvaney et al., 1996) as soon as possible. Dissolved organic C (DOC) was extracted with 2.0 M K_2SO_4 at 1:10 (w/v) and measured by a TOC analyzer (Multi N/C 2100, Jena, Germany).

2.3. N_2O sampling and measurement

The N_2O fluxes were measured in the morning between 8:00 and 10:00 every 3 to 5 days during the rice season. The N_2O fluxes were determined by the closed chamber method (Cai et al., 1997) The chamber (60 cm high and 23 cm inner diameter, made of polyvinylchloride) equipped with a dry battery driven fan and a gas sampling space tightened with the butyl rubber lids was used for sampling. Gas samples were collected by a 20 ml syringe at 0, 20, 40 and 60 min after the enclosure, respectively. The air temperature inside the chamber was monitored at the same time. Gas samples were injected into the pre-evacuated 18 ml vials which were equipped with butyl rubber lids and aluminum crowns for analyzing. The N_2O concentration was analyzed using a gas chromatograph (GC-14B, Shimadzu, Japan) with a stainless-steel column packed with 100-mesh Porapak Q and coupled with an electron capture detector (ECD). The injector and ECD detector were operated at 100 °C and 300 °C, respectively. The N_2O flux was calculated as $F = \rho \times h \times dc/dt \times 273/T$, where F is the N_2O emission flux, ρ is the density of N_2O at temperature T, h is the height of the closed chamber, dc/dt is the change in the N_2O concentration per unit in the closed chamber, and T is the Fahrenheit temperature inside the closed chamber.

2.4. qPCR analyses

The total DNA was extracted from 0.5 g of moist soil from each sample using a FastDNA® SPIN Kit for soil (MP Biomedical, LLC, OH, USA) according to the manufacturer's instructions. The extracted soil DNA was dissolved in 100 μl of tris-EDTA (TE) buffer, quantified by spectrophotometry, and stored at −20 °C until further use.

To quantify the abundance of microbial nitrogen-cycling functional genes during midseason drainage, a quantitative polymerase chain reaction (qPCR) was performed. Quantification of phylogenetic and functional genes *amoA* (bacteria and archaea), *nirK*, *nirS*, and *nosZ* was carried out using the gene-specific primers (archaea *amoA*, Arch-amoAF and Arch-amoAR (Chen et al., 2008); bacteria *amoA*, amoA-1F and amoA-2R (Chen et al., 2008); *nirK*, nirKF1aCu and nirKR3Cu (Throbäck et al., 2004); *nirS*, nirScd3aF and nirSR3cd (Throbäck et al., 2004); *nosZ*, nosLb and nosRb (Lópezgutiérrez et al., 2004). Serial tenfold dilutions (10^{-1} to 10^{-5}) of linearized plasmids containing cloned AOA *amoA*, AOB *amoA*, *nirK*, *nirS*, and *nosZ* genes were used to obtain standard curves. All sample and standard reactions were performed in triplicate using a CFX 96C 1000TM Thermal Cycler (Bio-Rad, Hercules, CA, USA), and average values were calculated. Each reaction was performed in 20 μl containing 2 μl of the total DNA template, 0.4 μl of each primer (10 mM), and 10 μl of SYBR Premix Ex Taq (Takara, Tokyo, Japan).

The PCR cycle started with 3 min at 95 °C, followed by a total of 40 cycles of 10 s at 95 °C, 30 s at 55 °C for the AOB *amoA* gene (57 °C for the AOA *amoA* and *nirS* genes and 58 °C for the *nirK* gene) and 30 s at 72 °C. For the *nosZ* gene, the PCR cycle started with 2 min at 94 °C, followed by a total of 30 cycles of 30 s at 94 °C, 30 s at 52 °C, and 45 s at 72 °C. The specificity of amplification was checked by melt curve observation. The PCR amplification efficiencies ranged from 85.0 to 100.7% with correlation coefficients (R^2) over 0.99 for all calibration curves.

2.5. Statistical analysis

Statistical procedures were performed with the software package SPSS 20.0 for Windows. Data were expressed as means with standard deviations of treatments. The letters above columns indicate statistical differences among treatments. Statistical differences were analyzed by one-way ANOVA and LSD testing. The level of $p < 0.05$ was considered statistically significant. The Spearman correlation coefficients were calculated to assess the association among N_2O emission rates, environmental parameters, soil properties, and functional gene abundance. A square correlation matrix was built using MATLAB.

3. Results

3.1. Rice plant growth

The rice plant height of each stage is presented in supplementary Fig. S1. At the tillering stage, the average plant height was measured under the following four conditions: eCO_2 + eTemp > eTemp > eCO_2 > aCO_2 + aTemp. However, at the beginning of the heading stage, elevated temperature had an adverse impact on plant growth. Elevated CO_2 concentration continued to promote plant growth throughout the season. The biochar effect of promoting plant height emerged gradually over time, and it was more obvious under eCO_2 and eTemp.

3.2. Soil chemical properties

The analysis results of soil moisture, pH, EC, and concentrations of dissolved organic carbon (DOC), NH_4^+-N, NO_2^--N, and NO_3^--N for the paddy soil tested during midseason drainage (30 DAT) are given in supplementary Table S1. Biochar addition did contribute to profound changes in the soil chemical properties.

Biochar addition significantly increased the soil moisture and pH at various CO_2 concentrations and air temperatures ($p < 0.05$). Compared to the ambient condition, simultaneously elevated CO_2 concentration and air temperature led to a significant reduction ($p < 0.05$) in the soil pH. The shift in soil pH between eeBC and eeCK was smaller than that of aaBC and aaCK. Either elevated CO_2 or elevated temperature resulted in a decline in soil EC. Under the ambient condition, aaBC had a significant lower soil EC than aaCK ($p < 0.05$).

Both the elevated temperature and elevated CO_2 significantly increased DOC concentrations in the tested soils ($p < 0.05$). There was a distinct trend for the DOC concentrations among control treatments: eeCK > eaCK > aeCK > aaCK. Biochar addition did not cause any obvious variation in the aTemp groups, but in the eTemp groups, the biochar soils (aeBC and eeBC) had lower DOC concentrations than the control soils (aeCK and eeCK) ($p < 0.05$). The group of eCO_2 and eTemp had the highest DOC concentrations among the four groups ($p < 0.05$).

During midseason drainage, the NH_4^+-N concentration of the eCO_2 and eTemp group was significantly lower than that of the aCO_2 and aTemp group ($p < 0.05$). Compared to the control soils under the four conditions, biochar amendment caused NH_4^+-N concentrations to decrease significantly by 15.5 to 21.6% ($p < 0.05$). The shifts in NO_2^--N and NO_3^--N concentrations induced by elevated CO_2 concentration or elevated air temperature were quite similar to that observed for NH_4^+-N concentrations. Biochar amendment had the opposite effect on the concentrations of soil NO_3^--N and NO_2^--N. Biochar soils had lower NO_3^--N concentrations but higher NO_2^--N concentrations in comparison to corresponding control soils under each condition. Compared to the corresponding control soils, there was a reduction of 6.6 to 18.0% in NO_3^--N concentrations and a significant rise ($p < 0.05$) of 22.4 to 54.8% in the NO_2^--N concentrations in biochar soils.

3.3. N_2O emission

The trend of N_2O fluxes during the rice season (Fig. 1) was similar for all treatments. The amount of N_2O emissions was generally low most of the time, and sometimes, it could not be detected. The peak emissions of N_2O fluxes occurred during midseason drainage from 23 DAT to 40 DAT with the highest flux at 30 DAT.

The cumulative N_2O emissions of 23–40 DAT are shown in Fig. 1. Compared to control treatments (85.0 μg kg^{-1} dws for aaCK, 0.34 μg kg^{-1} dws for aeCK, 25.5 μg kg^{-1} dws for eaCK, and −74.5 μg kg^{-1} dws for eeCK, respectively), biochar treatments had greater N_2O emissions (232.4 μg kg^{-1} dws for aaBC, 103.0 μg kg^{-1} dws for aeBC, 94.9 μg kg^{-1} dws for eaBC, and 24.3 μg kg^{-1} dws for eeBC, respectively) at various CO_2 concentrations and air temperatures. However, elevated CO_2 concentration or elevated air temperature reduced the amount of N_2O emitted from paddy soils with and without biochar during this period. The cumulative N_2O emissions of eeCK and eeBC were significantly lower than those of aaCK and aaBC ($p < 0.05$), respectively.

3.4. Abundance of functional microbial genes associated with nitrogen cycle

The abundance of ammonia-oxidizing archaea (AOA) (Fig. 2a) and ammonia-oxidizing bacteria (AOB) (Fig. 2b) was quantified by determining the numbers of archaeal and bacterial *amoA* gene copies. There was no statistically significant difference between the archaeal *amoA* gene data obtained under the ambient condition and that obtained under the elevated conditions. Archaeal *amoA* gene copy numbers were significantly higher with the addition of biochar ($p < 0.05$), rising from 8.44×10^6–1.08×10^7 to 1.51×10^7–2.28×10^7 gene copies per gram of dry soil.

Bacterial *amoA* gene copy numbers in the eCO_2 groups (1.19×10^7–1.91×10^7 gene copies per gram of dry soil) were lower than those in the aCO_2 groups (2.28×10^7–3.27×10^7 gene copies per gram of dry soil). The addition of biochar increased the numbers of bacterial *amoA* gene copies significantly by 16.6 to 36.5% ($p < 0.05$) with the exception of aeBC.

The ratio of AOA/AOB is shown in Fig. 2c. The average ratio remained in the range of 0.28 to 1.16, and it increased with CO_2 elevation. The AOA/AOB ratio in the eTemp groups was enhanced by biochar amendment from 0.28 and 0.61 to 0.76 and 1.16, respectively.

The abundance of nitrite-reducing bacteria and N_2O-reducing bacteria was quantified by determining the numbers of gene copies of *nirK* (Fig. 3a), *nirS* (Fig. 3b), and *nosZ* (Fig. 3c). Compared to the aCO_2 groups, the numbers of copies of the *nirK* gene in the eCO_2 groups decreased significantly by 60.2 to 74.8% ($p < 0.05$), from 9.15×10^7–1.34×10^8 to 2.30×10^7–4.95×10^7 gene copies per gram of dry soil. There was a notable increase in the abundance of the *nirK* gene in biochar soils. The addition of biochar in the eCO_2 groups increased the abundance of the *nirS* gene significantly ($p < 0.05$). Elevated CO_2 concentration or elevated air temperature did not affect the abundance of the *nosZ* gene. Compared to control soils, the numbers of copies of the *nosZ* gene increased significantly by 59.6 to 87.1% (from 2.03×10^7–2.84×10^7 to 3.50×10^7–5.18×10^7 gene copies per gram of dry soil) in biochar-amended soils.

The ratio of (*nirS* + *nirK*)/*nosZ* (Fig. 3d) could generally reflect a balance between the production and consumption of N_2O during denitrification. A (*nirS* + *nirK*)/*nosZ* ratio of 1 indicates equal numbers of nitrite reductase and nitrous oxide reductase gene copies per gram of dry soil (Harter et al., 2014). This ratio in eaCK and eeCK was significantly lower than that in aaCK and aeCK. However, the difference between the biochar soils and control soils under each condition with respect to the ratio was not statistically significant.

3.5. Correlation coefficients between N_2O emission rates and environmental properties

There were important interlinkages among N_2O emission rates, soil properties, and functional microbial genes on the nitrogen cycle (Fig. 4). N_2O emission rates not only had significant positive correlations with the abundance of the bacterial *amoA* gene (rho = 0.353, $p < 0.05$) and the *nirK* gene (rho = 0.352, $p < 0.05$), but also had a significant negative correlation with DOC concentration (rho = −0.383, $p < 0.05$). DOC concentration had significant correlations with the abundance of the *nirK* gene (rho = −0.819, $p < 0.01$), the *nirS* gene (rho = −0.365, $p <$

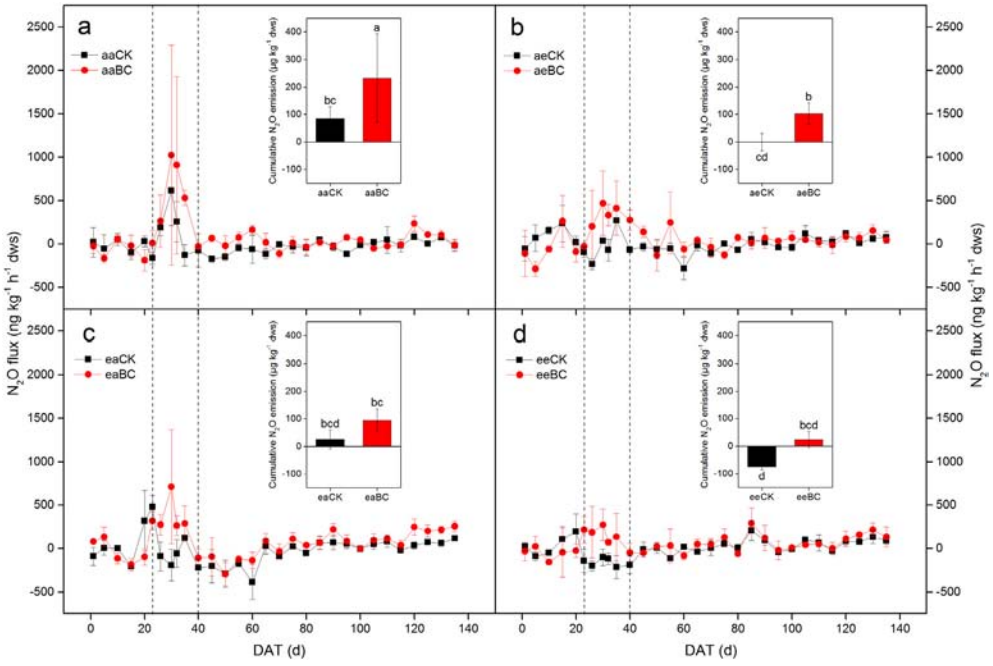

Fig. 1. N_2O emission flux during rice growth season at various atmospheric CO_2 concentrations and air temperatures with and without biochar (a. ambient; b. elevated temperature; c. elevated CO_2; d. simultaneously elevated air temperature and CO_2 concentration). The small inserted graphs show the cumulative N_2O emissions under various treatments from 23 DAT to 40 DAT. Black symbols represent data measured for control treatments, and red symbols represent data measured for biochar treatments. Different letters indicate significant differences between the eight treatments ($p < 0.05$).

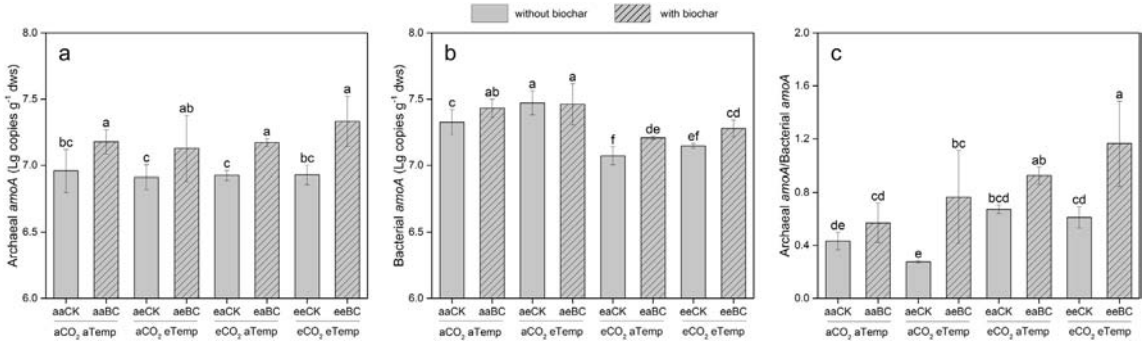

Fig. 2. Abundance of archaeal *amoA* gene (a) and bacterial *amoA* gene (b) and the ratio of archaeal *amoA* gene copies to bacterial *amoA* gene copies (c) in the rhizosphere soil with and without biochar during midseason drainage at various atmospheric CO_2 concentrations and air temperatures. Different letters indicate statistically significant differences among eight treatments ($p < 0.05$).

0.05), and the ratio of (*nirK* + *nirS*)/*nosZ* (rho = −0.470, $p < 0.01$). Soil moisture had a considerable impact on N_2O production and displayed high correlations with the NO_3^--N concentration (rho = 0.572, $p < 0.01$) and the gene abundance of archaeal and bacterial *amoA*, *nirK*, and *nosZ* (rho = 0.586, 0.356, 0.414, 0.563, respectively, $p < 0.05$). Soil pH also was a key factor in N_2O production and had significant correlations with the EC (rho = 0.550, $p < 0.01$), NO_3^--N concentration (rho = 0.640, $p < 0.01$), DOC concentration (rho = −0.364, $p < 0.05$). Furthermore, the abundance of bacterial *amoA* gene and *nirK* gene had significant positive correlations with NO_3^--N concentration (rho = 0.576, $p < 0.01$ and rho = 0.625, $p < 0.01$, respectively) and soil moisture (rho = 0.356, $p < 0.05$ and rho = 0.414, $p < 0.05$, respectively).

4. Discussion

Microbial nitrification and denitrification are generally the main biotic processes for N_2O production in soils (Bremner, 1997; Wrage et al., 2001). In contrast to the multiple mechanisms involved in N_2O formation, the only microbial sink for N_2O is reducing N_2O to N_2 by a nitrous oxide reductase encoded by the *nosZ* gene (Thomson et al., 2012). Soil mineral N, available organic C, soil moisture, and soil pH are considered as the essential factors that influence these pathways.

A peak flux appeared at 30 DAT. The air temperature during this period was maintained within the range of 20 to 33 °C. N_2O emission is usually stimulated to reach the maximum flux in the soil temperature range from 25 to 35 °C (Agehara and Warncke, 2005). This period, after urea application and during early midseason drainage, was the most active period for soil moisture change and nitrogen transformation. These conditions were optimal for N_2O production (Zou et al., 2009; Zou et al., 2007).

The cumulative emissions of N_2O with and without biochar during the peak period were suppressed under simultaneously elevated CO_2 concentration and air temperature. Elevated CO_2 concentration increased rice plant height and harvest biomass (Han et al., 2016), which agreed with the observations of previous studies (Cheng et al., 2006; Bhattacharyya et al., 2013; Pereira et al., 2013). The reduced soil mineral N concentrations could be attributed to competition from rice plant uptake under simultaneous elevation of CO_2 concentration and air temperature. The curtailment of substrates would consequently restrict N_2O production. Correlation coefficients revealed that there was

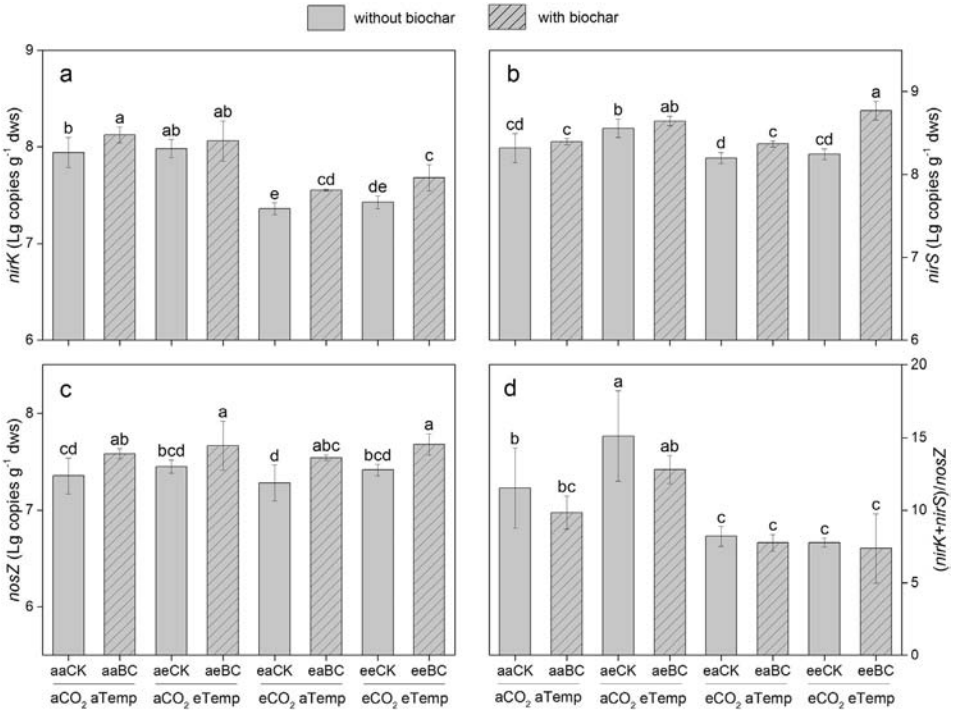

Fig. 3. Abundance of *nirK* gene (a), *nirS* gene (b), *nosZ* gene (c), and the ratio of (*nirS* + *nirK*)/*nosZ* (d) in the rhizosphere soil with and without biochar during midseason drainage at various atmospheric CO_2 concentrations and air temperatures. Different letters indicate statistically significant differences among eight treatments ($p < 0.05$).

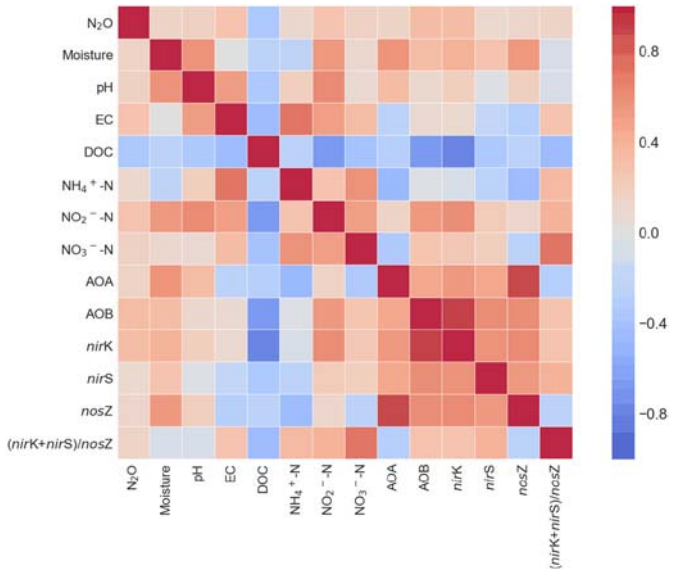

Fig. 4. Correlation matrices among N_2O emission rates, soil properties, and microbial functional genes. Red boxes represent positive correlation coefficients, and blue boxes represent negative correlation coefficients. Darker boxes represent high-correlation coefficients, and lighter boxes represent low-correlation coefficients.

a close association between N_2O emission rate and the abundance of the bacterial *amoA* gene and the *nirK* gene. The amount of N_2O generation from nitrification probably tended to diminish as the abundance of AOB decreased with simultaneous elevation in CO_2 concentration and air temperature. Similarly, N_2O produced by denitrification was likely to decline with the abundance of the *nirK* gene decreasing under these conditions. At the same time, below-ground C-input might increase with the enhancement in rice growth. Organic C availability is generally a restricting factor for denitrification in soils (Weier et al., 1993). The ratio of (*nirK* + *nirS*)/*nosZ* indicates that simultaneous elevation in CO_2 concentration and air temperature could facilitate N_2O reduction. Therefore, this condition could expedite denitrification by increasing DOC concentrations in paddy soils.

Many studies have suggested that biochar might have the ability to reduce N_2O emissions from soil under certain conditions. Many of these studies focused on the effect of biochar on soil denitrification. These studies have suggested that the mechanisms of this are mainly increasing soil pH (Obia et al., 2015), adsorbing N substrates (LeCroy et al., 2013; Singh et al., 2010; Angst et al., 2013), and reducing denitrifying bacteria (Wang et al., 2017). Recent evidence has revealed that biochar amendment could facilitate N_2O reduction by promoting the *nosZ* gene (Harter et al., 2014; Van Zwieten et al., 2014; Wang et al., 2013). In this study, biochar addition did significantly increase soil moisture, pH, and *nosZ* gene abundance ($p < 0.05$). The nitrate concentrations decreased, while the abundance of the *nirK* and *nirS* genes increased in biochar soils. Hence, biochar amendment was believed to have an impact on denitrification. Besides, *nosZ* gene abundance had positive correlations with soil moisture. Increased soil moisture can provide relatively anaerobic conditions with less O_2 availability in the rhizosphere, which is beneficial to N_2O reduction via *nosZ*-bearing denitrifiers (Weier et al., 1993).

Despite this, biochar amendment stimulated N_2O emission from paddy soils in this study. There was no statistically significant difference between the (*nirS* + *nirK*)/*nosZ* ratios of biochar soils and control soils, which indicates that biochar may have little effect on soil N_2O produced by denitrification. The products ratio (N_2O/N_2) of denitrification generally decreases with increased pH values (Weier and Gilliam, 1986; Bergaust et al., 2010; Liu et al., 2010; Qu et al., 2014; Raut et al., 2012). If the total amount of N_2O emitted was from the denitrification pathway, biochar treatments should produce less N_2O emissions than the corresponding controls. Apparently, the results showed otherwise. Instead of denitrification, nitrification was assumed to contribute to the biochar-induced discrepancy in N_2O emissions. The ammonium concentration was much higher than the nitrate concentration for each treatment (Table S1). After fertilizer was added, urea hydrolysis was believed to provide the substrate for nitrification which activity was proved to be stimulated by urea fertilization in paddy soils (Jiang et al., 2015). During midseason drainage, the soil moisture was maintained within the range of 45.60 to 50.84%. Previous studies have reported that nitrification could act as the driving source of N_2O generation when soil water content was maintained within the range of 30 to 70% WFPS (water-filled pore space) (Braker and Conrad, 2011; Huang et al., 2014). Ammonia oxidation was proved to contribute >80% of total N_2O emissions from NH_4^+-N-fertilized arable soil at 45% WFPS (Well et al., 2008).

The abundance of AOA and AOB showed an obvious increase in biochar soils with a decrease in ammonium concentrations. This suggests that ammonia oxidation could be promoted in biochar- amended soils. Enhanced ammonia oxidation probably implies greater N_2O generation through the nitrification pathway. Evidence regarding the effects of biochar on soil nitrification has been accumulating in recent years. Several studies have reported that biochar incorporation could stimulate ammonia-oxidizer populations in temperate arable soil (Prommer et al., 2014) or wheat-cultivated soils (Liu et al., 2017) and thus might accelerate gross nitrification rates (Prommer et al., 2014). Under certain conditions, biochar addition would increase N_2O emissions produced by nitrification-mediated pathways (Sánchez-García et al., 2014). The results obtained in this study are in line with these findings.

Biochar-induced changes in soil pH and moisture could play key roles in regulating N_2O production during nitrification. An increase in soil pH is likely to provide the alkalinity needed for the conversion of NH_4^+ into NH_3, which is the bioavailable substrate for ammonia monooxygenase (Suzuki et al., 1974). Accelerating the rate-limiting step (ammonia oxidation) may be an important reason that biochar promotes microbial nitrogen cycles in paddy soils. Hydroxylamine, an intermediate of ammonia oxidation, is a type of precursor for N_2O formation (Conrad, 1996). The proportion of N_2O derived from hydroxylamine is considered to have a positive correlation with soil pH (Heil et al., 2015). Regarding substrate concentrations, compared to corresponding controls, biochar soils had higher nitrite but lower ammonium and nitrate concentrations. The stability of nitrite in soil is sensitive to soil pH. N_2O could be emitted from the decomposition of nitrite in acidic and neutral soils (Bremner, 1997; Cleemput and Samater, 1996). The NO_2^--N concentrations had positive correlations with soil moisture and soil pH. Increased soil moisture in biochar soils may not be beneficial for the oxidation of nitrite to nitrate. Enriched NH_3 induced by increased soil pH is thought to inhibit the activity of nitrite-oxidizing bacteria (Smith et al., 1997; Burns et al., 1996). With respect to the gene abundance of *amoA*, *nirK*, and *nirS*, nitrite accumulation in biochar soils probably results from promoted ammonia oxidation.

However, it was notable that the soil pH significantly decreased at simultaneously elevated CO_2 concentrations and air temperatures. Furthermore, the pH gap between biochar soils and control soils under this condition became narrower than that at ambient CO_2 concentration and air temperature. It can be inferred that biochar's liming effect in paddy soils is probably neutralized by simultaneous elevation of CO_2 concentration and air temperature. As a result, the promoting effect of biochar on ammonia oxidation might become weakened, which is likely to attenuate the stimulating effect of biochar on N_2O emissions from paddy soils.

5. Conclusion

Biochar amendment increased N_2O emissions from urea-fertilized paddy soils during midseason drainage at ambient CO_2 concentration

and air temperature. Biochar amendment might facilitate N_2O reduction via denitrification by increasing soil moisture and *nosZ* gene abundance. Moreover, it could stimulate ammonia oxidation-induced N_2O production during nitrification with increasing soil pH and *amoA* gene abundance. However, the reduced mineral N concentrations and increased DOC concentrations would restrict the amount of N_2O emitted from paddy soils with and without biochar at simultaneously elevated CO_2 concentrations and air temperatures. Moreover, the neutralized liming effect of biochar was probably related to the attenuated stimulating effect of biochar on N_2O emissions at simultaneously elevated CO_2 concentration and air temperature. Soil pH-induced changes in ammonia oxidation should be a concern when controlling N_2O emissions with biochar amendment in urea-fertilized paddy soils at elevated CO_2 concentration and air temperature.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant number 41571241); the Natural Science Foundation of Zhejiang Province (grant number LZ15D030001, LY14C010004). We greatly thank Chen Chen, Qi Liu, Cheng Wang and Weijing Zhu for help with manuscript revision, and greatly appreciate editors and reviewers for constructive comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.02.046.

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